The independent role of the aortic root ganglionated plexi in the initiation of atrial fibrillation: An experimental study

Hong-Tao Wang, MD,[a] Zhi-Li Li, MD,[a] Bo-Yuan Fan, MD,[a] Fei-Fei Su, MD,[a] Jin-Bo Zhao, MD,[b] Jun Ren, MD, PhD,[c] and Qiang-Sun Zheng[a]

Objective: The major atrial ganglionated plexi (GP) can initiate atrial fibrillation alone without any contribution from the extrinsic cardiac nervous system. However, if stimulation of the ventricular GP, especially the aortic root GP, can provoke atrial fibrillation (AF) alone is unknown. Our study was designed to investigate the independent role of aortic root GP activity in the initiation of AF.

Methods: In 10 Langendorff-perfused canine hearts, the atrial effective refractory period, pulmonary vein effective refractory period, and percentage of AF induced were measured at baseline and during aortic root GP stimulation.

Results: Stimulation of the aortic root GP shortened the atrial effective refractory period from 128 ± 10 ms at baseline to 103 ± 15 ms ($P < .05$) and shortened the pulmonary vein effective refractory period from 139 ± 14 ms to 114 ± 15 ms ($P < .05$). Furthermore, the percentage of AF induced in the 10 isolated hearts increased from 10% at baseline to 90% during aortic root GP stimulation ($P < .05$).

Conclusions: In Langendorff-perfused canine hearts, stimulation of the aortic root GP provokes AF in the absence of any extrinsic cardiac nerve activity. The aortic root GP is an important element in the intrinsic neuronal loop that can increase the risk of AF in isolated heart models. (J Thorac Cardiovasc Surg 2014;148:73-6)

ACD

Video clip is available online.

The intrinsic cardiac nervous system mainly consists of some major epicardial ganglionated plexi (GP), including the atrial GP and the ventricular GP. The atrial GP mainly consist of the superior left GP, the anterior right GP, the inferior right GP, the inferior left GP, and the GP within the ligament of Marshall.[1] It is well known that the atrial GP play an important role in both the initiation and/or the maintenance of atrial fibrillation (AF).[2-4] However, there is little information on the effect of the ventricular GP on the initiation and/or the maintenance of AF. The ventricular GP include the aortic root GP, the anterior descending GP, the posterior descending GP, the right acute marginal GP, and the obtuse marginal GP. These GP are thought to mainly influence the function of the ventricles and/or coronary arteries.[1] However, a study by Jennifer and colleagues[5] demonstrated that preservation of the human anterior epicardial GP (which has the same location as the canine aortic root GP) during coronary artery bypass surgery decreased the incidence of postoperative AF. This result indicated that the ventricular GP, especially the aortic root GP, might be important in the initiation of AF. Therefore, this study was designed to investigate if the ventricular GP, especially the aortic root GP, can trigger AF in the absence of extrinsic cardiac nervous activity (ECNA). To this end, the Langendorff-perfused heart model was employed to exclude the influence of extrinsic cardiac nervous system and to investigate if stimulation of the aortic root GP may provoke AF.

METHODS

The Langendorff-Perfused Heart Model

A total of 10 mongrel dogs of either sex weighing between 15 and 23 kg were anesthetized with 30 mg/kg sodium pentobarbital and ventilated with room air (model DDH-1, No. 3529 factory of PLA). After the chest was opened through a median sternotomy, heparin (8000 U) was injected into the left ventricle. The hearts were rapidly excised and immersed in cold Tyrode's solution and connective tissue was removed. The isolated hearts were then connected to a Langendorff-perfusion apparatus (Radnoti Company, Monrovia, Calif). To ensure a leak-proof connection, the aorta and perfusion cannula were connected by a custom adapter, and a gelatin sponge was placed in the gap between the cannula and the adapter. Then, the hearts were perfused with oxygenated Tyrode's solution at 37.5°C. The Tyrode's solution had the following ionic concentrations: dextrose 5.5 mmol/L, sodium chloride 125 mmol/L, potassium chloride 4.5 mmol/L, sodium phosphate 1.8 mmol/L, calcium chloride 2.7 mmol/L, magnesium chloride 0.5 mmol/L, and sodium bicarbonate 24 mmol/L. The hearts were immersed in a 500-mL beaker filled with Tyrode's solution, placed in a thermostatic water bath (Westingarea Company, Shanghei, China), and maintained at a constant

From the Division of Cardiology,[a] and Division of Thoracic Surgery,[b] Tangdu Hospital, Xi'an, Shaanxi, China; and Division of Pharmaceutical Sciences,[c] School of Pharmacy, University of Wyoming College of Health Sciences, Laramie, Wyo.

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Address for reprints: Qiang-Sun Zheng, Division of Cardiology, Tangdu Hospital, Xinsi Rd, Baqiao District, Xi'an, Shaanxi, 710038, China (E-mail: qiangsun_tdxn@hotmail.com).

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Abbreviations and Acronyms

AERP = atrial effective refractory period
AF = atrial fibrillation
ECNA = extrinsic cardiac nervous activity
GP = ganglionated plexi
ICNA = intrinsic cardiac nerve activity
PVERP = pulmonary vein effective refractory period

temperature of 37.5°C (Video 1). Cardiac compression was applied during the perfusion process. The hearts were first perfused at 30 mL/minute and then at 15 mL/minute, and manual cardiac compression was applied frequently during perfusion until the contractile force became normal and the heart rate was stable at approximately 120 bpm. If heart failure occurred after prolonged perfusion due to cellular edema, 20% mannitol was used to reduce edema. All animals received humane care, and the study protocol complied with the institution's guidelines on the care and use of animals.

Measurement of Refractory Periods

The pulmonary vein effective refractory period (PVERP), and atrial effective refractory periods (AERP) were measured at baseline and during aortic root GP stimulation using the extrastimulus technique (basic cycle length 400 ms, final extrastimulus decrement 5 ms, and pacing stimuli at twice the diastolic pacing threshold). All stimuli were monitored on a 64-channel electrophysiologic recorder (Henan Huanan Medical Science & Technology Co, Zhengzhou, Henan, China). The longest coupling interval that did not capture the atrium was defined as the AERP.[6] The AERP was measured at the left atrial appendage and right atrial appendage. The PVERP was measured at the proximal portion of the 4 pulmonary veins using the same protocol as described above.

Stimulation of the Aortic Root GP

The aortic root GP was located in the fat surrounding the aortic root (Figure 1). To stimulate the neurons in the aortic root GP, a custom electrode with 8 metal electrode heads was used. The following parameters were used: a stimulation frequency of 20 Hz, pulse duration of 0.1 ms, train duration of 50 ms, and voltage of 0.6 to 2.4 V (programming stimulator model 5329, Astro Med Inc, West Warwick, Mass). This high frequency stimulation did not excite the atrium, which would directly cause atrial acceleration.[7]

Inducibility of AF

AF was induced during aortic root GP stimulation by programmed electrical stimulation of the left atrial appendage sequentially at basic cycle lengths of 400 and 300 ms with up to 2 extrastimuli (S_3). All stimuli were monitored on a 64-channel electrophysiologic recorder (Henan Huanan Medical Science & Technology Co, Zhengzhou, Henan, China). The first extrastimulus (S_2) was introduced with an S_1 to S_2 interval of 30 ms longer than the atrial effective refractory period and the coupling interval was decreased in 10-ms decrements (Figure 2). If S_2 failed to induce arrhythmia, a second extrastimulus (S_3) was introduced while the S_1 to S_2 interval was set at 80% of the basic cycle length, and the coupling interval of S_3 was reduced in 10-ms decrements. Sustained AF was defined as irregular repetitive atrial responses lasting >20 seconds.[8] A 10-minute recovery period was allowed between the spontaneous termination of AF and the next pacing sequence.[9] The percentage of hearts (n = 10) in which AF was induced by programmed electrical stimulation was calculated.

Statistical Analysis

All data are expressed as mean ± standard deviation. The AERP and PVERP were compared using least significant difference *t* test. A χ^2 test was used to evaluate the inducibility of AF.

RESULTS

Effect of Epicardial GP Stimulation on Atrial Electrophysiologic Properties

Aortic root GP stimulation altered the atrial electrophysiologic properties. The AERP was shortened from 128 ± 10 to 103 ± 15 ms at baseline (n = 10) ($P < .05$) (Table 1). Compared with the baseline value, the PVERP was significantly shortened from 139 ± 14 to 114 ± 15 ms ($P < .05$) (Table 1).

Effect of Aortic Root GPs Stimulation on AF Inducibility

At baseline, AF was induced by programmed stimulation in only 1 heart (10% induction rate) (Table 2). However, programmed stimulation during aortic root GP stimulation induced AF in 9 hearts (90% induction rate) and this was a significant increase compared with baseline

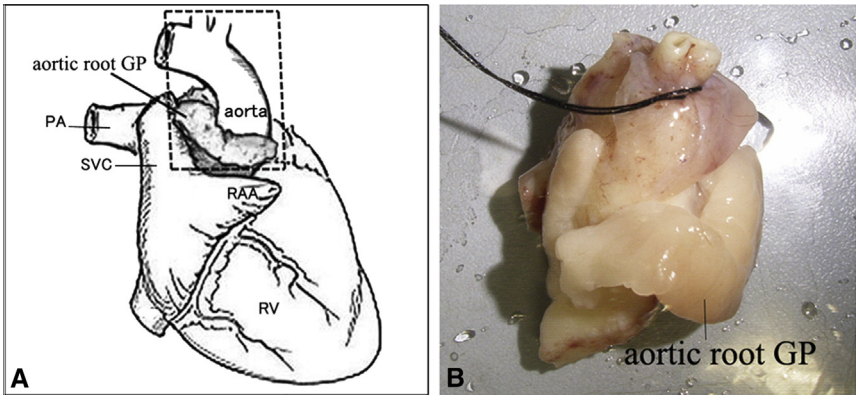

FIGURE 1. Location of canine aortic root ganglionated plexi (*GP*) (*front view*). A, The aortic root GP surrounded the aortic root and was connected with the aorta by the mesangial ligament. Major neuron cells that projected to the ventricles were embedded in this GP. B, The anatomic specimen of canine aortic root GP (the section surrounded by *dashed lines* in Figure 1, *A*). *PA*, Pulmonary artery; *SVC*, superior vena cava; *RAA*, right atrial appendage; *RV*, right ventricle.

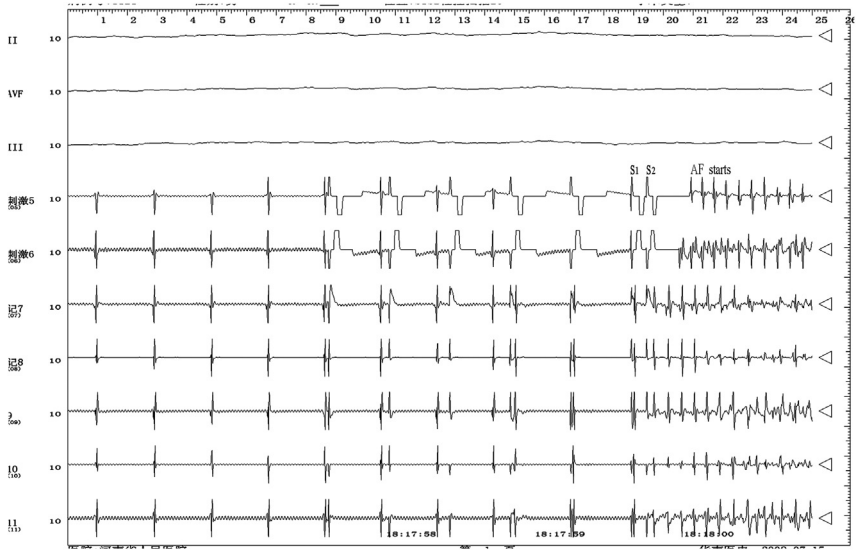

FIGURE 2. Induction of atrial fibrillation (*AF*) from the left atrial appendage (*LAA*) by programmed electrical stimulation. Premature stimulation at the LAA was used to induce AF with basic cycle lengths of 400 and 300 ms and up to 2 extrastimuli (S_3). AF began when S_2 was decreased to 110 ms. Channels 5 and 6 are the stimulating channels, and channels 7 through 11 are the recording channels.

(Table 2) ($P < .01$). This result demonstrated that stimulation of the aortic root GP may provoke AF in the absence of ECNA.

DISCUSSION

Both ECNA and intrinsic cardiac nerve activity (ICNA) may provoke AF. In general, these 2 nervous systems seem to participate jointly in the initiation of AF. Choi and colleagues[10] claimed that the ICNA mediated by the major atrial GP may function independently of inputs from ECNA, indicating an important and independent role of ICNA in cardiac arrhythmogenesis. Tan and colleagues[11] demonstrated a critical role of the ICNA in the initiation of AF. They claimed that both sympathetic and vagal activation preceded the onset of paroxysmal AF in the majority of episodes. In the remaining episodes, there were no apparent ECNA triggers for atrial tachyarrhythmia. These previous studies suggested that the atrial GP may independently provoke AF. Therefore, many investigators have ablated the atrial GP to try to decrease the recurrence rate of AF. Scherlag and colleagues[12] further stated that ablation of only the GP in cardiac fat pads may result in higher success rates and may reduce damage of healthy myocardium. Po and colleagues[2] claimed that ablation of the left atrial GP after pulmonary vein antrum ablation decreased the recurrence rate of AF 12 months later. Furthermore, they postulated that this late benefit may result from destruction of autonomic neurons in the GP that cannot regenerate. Despite these findings, the cure rate of AF is sill low when radiofrequency ablation is used to treat AF, especially in patients with persistent AF. In addition, the long-term outcome of GP ablation is still controversial. Oh and colleagues[13] demonstrated that ablation of the right pulmonary vein and inferior vena cava–left atrial GP decreased the inducibility of AF. However, the inducibility of AF returned to preablation levels after 4 weeks. Lall and colleagues[14] suggested that residual autonomic effects were still present in the atria even after a complete Cox-maze procedure. Sakamoto and colleagues[15] claimed that ablation of 4 human atrial GPs significantly reduced atrial vagal innervation, whereas restoration of vagal effects at 4 weeks indicated early atrial reinnervation. Neural remodeling was a possible mechanism for the increased response at 4 weeks. These studies suggest that GP ablation provides

TABLE 1. Electrophysiologic changes during aortic root ganglionated plexi stimulation

	AERP (ms)	PVERP (ms)
Baseline	128 ± 10	139 ± 14
Stimulation	103 ± 15*	114 ± 15*

AERP, Atrial effective refractory period; *PVERP*, pulmonary vein effective refractory period. *$P < .05$ versus baseline.

TABLE 2. Comparison of atrial fibrillation inducibility before and during ganglionated plexi stimulation (N = 10)

	AF inductions	
	n	%
Baseline	1	10
Stimulation	9	90*

Programmed stimulation was performed to provoke AF before and during stimulation of the aortic root ganglionated plexi. The percentage of AF induced in the 10 isolated hearts increased significantly during ganglionated plexi stimulation compared with baseline. *AF*, Atrial fibrillation. *$P < .05$ versus baseline.

only short-term gains in the treatment of AF. However, little is known about whether the ventricular GP can trigger AF in the absence of ECNA.

From an anatomic viewpoint, at least 7 GPs are located in canine epicardium, including 3 ventricular GPs.[16] Several studies have investigated the role of the aortic root GP. James[17] demonstrated that stimulation of the aortic GP raised blood pressure. Butler and colleagues[18] found no heart rate change in response to electrical stimulation of the ventricular GP. Our study focused on the electrophysiologic effects of GP stimulation on the atrium, and the major finding was that stimulation of the aortic root GP is capable of independently provoking AF in the absence of ECNA. Although we did not simulate the atrial GP in our study, based on our previous work, it would appear that the aortic root GP has a similar effect on the initiation of AF as the atrial GP. Given that the influence of ECNA was completely excluded in the Langendorff-perfused heart model, this result suggested that a local neuronal loop consisting of connections between the aortic root GP and other atrial GP may play a key role in the induction of AF. It would also appear that the aortic root GP is an important element in this loop.

Clinical Implications

Epicardial GP ablation has been widely used in the clinical setting as a supplement to pulmonary vein isolation. However, the recurrence rate of AF, especially persistent AF, is still high. One possible reason for this disappointing outcome is insufficient ablation of the autonomic nerves. The results of our study indicate that the ventricular GP, especially the aortic root GP, might be ablated as well as the atrial GP for AF control.

It should be noted that there are differences in the number of GPs and in the distribution of nerves between dogs and humans. The number of nerves is smaller in dogs than in humans. In addition, about 70% of all intrinsic ganglia supply the sinus node in dogs, whereas most of the ganglia supply the atrioventricular node in humans.[19] Therefore, caution should be used in extrapolating these findings to the clinical setting for humans.

Study Limitations

First, cardiac enlargement and heart failure cannot be avoided after long-time perfusion, especially after 2 or 3 hours. Second, further studies should investigate the other 4 ventricular GPs to better characterize the relationship between the ventricular GP and AF.

CONCLUSIONS

In Langendorff-perfused canine hearts, stimulation of the aortic root GP provokes AF in the absence of any extrinsic cardiac nerve activity. The aortic root GP is an important element in the intrinsic neuronal loop that can increase the risk of AF in isolated heart models.

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References

1. Yuan BX, Ardell JL, Hopkins DA, Losier AM, Armour JA. Gross and microscopic anatomy of the canine intrinsic cardiac nervous system. *Anat Rec*. 1994;239:75-87.
2. Po SS, Nakagawa H, Jackman WM. Localization of left atrial ganglionated plexi in patients with atrial fibrillation. *J Cardiovasc Electrophysiol*. 2009;20:1186-9.
3. Hou Y, Scherlag BJ, Lin J, Zhang Y, Lu Z, Truong K, et al. Ganglionated plexi modulate extrinsic cardiac autonomic nerve input: effects on sinus rate, atrioventricular conduction, refractoriness, and inducibility of atrial fibrillation. *J Am Coll Cardiol*. 2007;50:61-8.
4. Lu Z, Scherlag BJ, Lin J, Niu G, Fung KM, Zhao L, et al. Atrial fibrillation begets atrial fibrillation: autonomic mechanism for atrial electrical remodeling induced by short-term rapid atrial pacing. *Circ Arrhythm Electrophysiol*. 2008;1:184-92.
5. Jennifer E, Cummings M, Inderjit G, Dery M, Biblo LA, Quan KJ. Preservation of the anterior fat pad paradoxically decreases the incidence of postoperative atrial fibrillation in humans. *J Am Coll Cardiol*. 2004;6:994-8.
6. Wang HT, Li J, Hong CM, Liu X, Shang F, He Y, et al. Epicardial ablation of right pulmonary artery ganglionated plexi for the prevention of atrial fibrillation originating in the pulmonary veins. *J Electrocardiol*. 2010;43:367-72.
7. Furukawa Y, Wallick DW, Carlson MD. Prolongation of cardiac cycle length attenuates negative dromotropic response to selective vagal stimuli. *J Auton Nerv Syst*. 1989;28:43-50.
8. Schauerte P, Scherlag BJ, Pitha J, Scherlag MA, Reynolds D, Lazzara R, et al. Catheter ablation of cardiac autonomic nerves for prevention of vagal atrial fibrillation. *Circulation*. 2000;102:2774-9.
9. Sticherling C, Oral H, Horrocks J, Chough SP, Baker RL, Kim MH, et al. Effects of digoxin on acute, atrial fibrillation-induced changes in atrial refractoriness. *Circulation*. 2000;102:2503-8.
10. Choi EK, Shen MJ, Han S, Kim D, Hwang S, Sayfo S, et al. Intrinsic cardiac nerve activity and paroxysmal atrial tachyarrhythmia in ambulatory dogs. *Circulation*. 2010;24:2615-23.
11. Tan AY, Zhou S, Ogawa M, Song J, Chu M, Li H, et al. Neural mechanisms of paroxysmal atrial fibrillation and paroxysmal atrial tachycardia in ambulatory canines. *Circulation*. 2008;118:916-25.
12. Scherlag BJ, Patterson E, Po SS. The neural basis of atrial fibrillation. *J Cardiovasc Electrophysiol*. 2006;39(Suppl):S180-3.
13. Oh S, Zhang Y, Bibevski S, Marrouche NF, Natale A, Mazgalev TN. Vagal denervation and atrial fibrillation inducibility: epicardial fat pad ablation does not have long-term effects. *Heart Rhythm*. 2006;3:701-8.
14. Lall SC, Foyil KV, Sakamoto S, Voeller RK, Boineau JP, Damiano RJ Jr, et al. Pulmonary vein isolation and the Cox maze procedure only partially denervate the atrium. *J Thorac Cardiovasc Surg*. 2008;135:894-900.
15. Sakamoto S, Schuessler RB, Lee AM, Aziz A, Lall SC, Damiano RJ Jr. Vagal denervation and reinnervation after ablation of ganglionated plexi. *J Thorac Cardiovasc Surg*. 2010;139:444-52.
16. Ardell JL. Structure and function of mammalian intrinsic cardiac neurons. In: Armour JA, Ardell JL, eds. *Neurocardiology*. New York, NY: Oxford University Press; 1994:95-114.
17. James TN. Combinatorial roles of the human intertruncal plexus in mediating both afferent and efferent autonomic neural traffic and producing a cardiogenic hypertensive chemoreflex. *Prog Cardiovasc Dis*. 2004;46:539-72.
18. Butler CK, Smith FM, Cardinal R, Murphy DA, Hopkins DA, Armour JA. Cardiac responses to electrical stimulation of discrete loci in canine atrial and ventricular ganglionted plexi. *Am J Physiol*. 1990;259:H1365-73.
19. Pauza DH, Skripka V, Pauziene N. Morphology of the intrinsic cardiac nervous system in the dog: a whole-mount study employing histochemical staining with acetylcholinesterase. *Cells Tissues Organs*. 2002;172:297-320.